

12/20/01

Experimental plans for asthma study:**Purpose**

Evaluate the efficacy of anti-C5 (BB5.1) on asthma mouse model.

Animals

BALB/c mice (8-12 weeks old) are purchased from the Jackson laboratory and maintained at Alexion's pathogen free facility.

Antigen sensitization and challenge

BALB/c mice will be sensitized on day 1 and 14 by intraperitoneal injection of 20ug OVA, (Grade V, Sigma) emulsified in 2mg aluminum hydroxide (Alum inject, Pierce) in a total volume of 100 ul. All mice will be challenged daily with OVA for 10 minutes via the airways (1% OVA in saline) for 3 days (day 28, 29, and 30), using ultrasonic nebulization. In day 32, mice will be provoked with OVA (5% in saline) for 10 minutes to elicit an antigen-induced early-phase reaction (EPR) and late-phase reaction (LPR). Negative control mice will be sensitized with alum only and challenged with saline instead of OVA.

1 Plan one:**(1) prophylactic treatment**

Beginning on day 25 the experimental groups will be injected with either anti-C5 (40mg/kg, S.C. injection in 0.2 ml saline) or Dex(2mg/kg, S.C. injection in 0.2 ml saline) also day 29 and 31. After 5 hours of 5% OVA provocation, mice will be sacrificed and bronchoalveolar lavage (BAL) will be performed by instilling of 1ml ice-cold PBS through the tracheal cannula, followed by gentle aspiration.

The BAL fluid will be collected.

1. Cell differential counts.

2. IgE measurement.

3. Analysis of cytokines Cell differential counts. IL-4, IL-5, IL-13 and TGF-beta

After BAL, lungs will be inflated with 10% buffered formalin (1ml), removed from chest cavity and fixed in 10% formalin at least 24h then stained with hematoxylin and eosin and examined by light microscopy for evaluation of the severity of inflammation.

(2) therapeutic treatment

In day 35, mice will be provoked with 5% OVA again, then the mice will be injected with anti-C5 (40mg/kg, I.V. injection in 0.2 ml saline) or Dex(2mg/kg, I.V. injection in 0.2 ml saline). After 5 hours, the mice will be done at both BAL and histology as the same as above.

2 Plan two:

In day 35, Baseline lung resistance and methacholine induced AHR will be measured in OVA sensitised mice and non-sensitised mice. By increasing doses of intravenous methacholine (5, 15, 45, 137, 411 ug/kg), we will make invasive measurement of tracheal pressure in 5-min interval (see detail in our invasive method). Mice can be treated with BB5.1 in a dose of 40mg/kg 24 hours, 8 hours, 4 hours, and 2 hours before methacholine challenge. Control group will treated with 135.8 and PBS.

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